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Serum Concentrations of IL-17 and IL-22 as Possible Biomarkers of Hepatocellular Carcinoma

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Abstract

Background: In the majority of patients, Hepatocellular Carcinoma (HCC) develops from liver cirrhosis, but the developed cirrhosis is not necessary for the development of HCC. At this time, there are no reliable blood-based biomarkers that could identify the malignant transformation in liver parenchyma. We examined whether serum concentrations of IL-17 and IL-22 proteins could be used to point out patients with liver disease, fibrosis, or cirrhosis who have possibly developed HCC.

Methods: This study included 198 patients and 28 Healthy Controls (HC). The study included 33 patients with HCC, 51 with liver cirrhosis and no morphological signs for HCC, 43 with viral hepatitis B or C, 50 with autoimmune liver disease, and 21 with non-alcoholic fatty liver disease without cirrhosis. Concentrations of IL-17 and IL-22 in sera were determined by Enzyme-Linked Immunosorbent Assay (ELISA).

Results: Serum concentrations of IL-17 and IL-22 were significantly higher in patients with liver diseases than in control patients. Patients with liver cirrhosis had significantly higher IL-17 and IL-22 concentrations than other liver diseases. Notably, both IL-17 and IL-22 were significantly higher in patients with liver cirrhosis than those with hepatocellular carcinoma. IL-17 and IL-22 were significantly positively correlated in all types of liver diseases except hepatocellular carcinoma.

Conclusion: Based on the differences in concentration of IL-17 and IL-22 in sera of patients with liver diseases and healthy controls, and then patients with LC and HCC, those interleukins are emerging as possible candidates for biomarkers that could be used to identify an occurrence of HCC.

Keywords: Liver; Inflammation; Cirrhosis; Hepatocellular carcinoma; Interleukin-17; Interleukin-22; Croatian caucasian population

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Introduction

Liver Cirrhosis (LC) is responsible for more than 1,000,000 deaths annually worldwide [1]. It can be induced by a viral infection, alcohol toxicity, non-alcoholic fatty liver disease/steatohepatitis, autoimmune hepatitis, primary sclerosing cholangitis, or primary biliary cholangitis [2]. If not resolved, chronic liver inflammation can lead to liver fibrosis, cirrhosis, and hepatocellular carcinoma [3-5]. Hepatocellular Carcinoma (HCC) accounts for most primary liver cancers, ranking sixth by incidence and the fourth most common cause of death worldwide [6]. It comprises 75% - 85% of cases of liver carcinoma [7].

The majority of newly diagnosed HCC cases occur in patients with LC, suggesting a progression of LC into HCC as the key etiological factor of HCC. Although uncommon, chronic liver diseases

can progress into HCC even without LC [8]. However, early clinical diagnosis of HCC occurrence is challenging. Blood-based biomarkers of such progression to HCC, as a form of liver liquid biopsy, which are clinically very convenient for frequent monitoring, are lacking.

The interplay between IL-17 and IL-22 is essential in maintaining tissue homeostasis [9]. The relevance of their role in inflammation became more evident after the discovery of Th17 cells as an independent line of T-helper cells [10,11], which secrete both IL-17 and IL-22 cytokines. The stimulus for their secretion still needs to be clarified, but in humans, unlike mice models, they are not always synergistically secreted [12,13]. In general, IL-17 is mostly a pro-inflammatory cytokine that protects against infection by enhancing the epithelial release of antimicrobial peptides, granulopoiesis, and neutrophil accumulation in peripheral tissues, and IL-22 is a homeostatic cytokine preserving the integrity of boundary organs and tissues, and only occasionally exerts a pro-inflammatory role [13,14]. IL-17 and IL-22 are considered to be tissue-signaling cytokines since they affect mostly cells from the solid tissues [12,15,16]. The distribution of their receptors diverges considerably. In humans, the receptor complex of IL-17 is widely expressed on epithelial, mesenchymal, and hematopoietic cells [13,17]. IL-17 and Th17 cells have been associated with pro-inflammatory and profibrotic conditions in various organs, such as the liver or skin [18,19].

Th17 role in cancer progression is rather complex, ranging from antitumorigenic to tumorigenic effects [20,21].

We designed this study to analyze serum concentrations of cytokines IL-17 and IL-22 in patients with various liver diseases. Our aim was to test their relevance and to test theses cytokines as potential biomarkers of HCC development in patients with liver cirrhosis.

Identifying the transition from liver fibrosis/cirrhosis to HCC is a significant problem for clinicians due to the lack of appropriate diagnostics tools, including reliable blood-based biomarkers [22,23].

Materials and Methods

Study design, patients, and samples

We conducted a prospective study on patients with liver disease from 2014 to 2019.

The diagnosis of liver lesion etiology was determined according to the EASL guidelines for treating hepatocellular carcinoma [24], liver cirrhosis [25], hepatitis B [26], and C [27], autoimmune liver diseases (autoimmune hepatitis, primary biliary cholangitis, primary sclerosing cholangitis) [28-30], and non-alcoholic fatty liver disease [31,32]. Peripheral blood samples were obtained from the patients during routine laboratory testing at the end of the diagnosing process, centrifuged, and then stored at -80 degrees Celsius for subsequent analysis. Aspartate-aminotransferase (AST), Alanine-aminotransferase (ALT), albumin, and Alpha-fetoprotein (AFP) were determined by the standard procedure at Clinical Hospital Centre Zagreb. Liver stiffness was measured by shear wave transient elastography (Fibroscan Touch 502, Echosence, France), and steatosis was measured by Continuous Attenuation Parameter (CAP), also done by Fibroscan Touch 502, Echosence France. Child-Pugh status was calculated from clinical data and laboratory findings obtained in routine laboratory evaluation [33]. All patients were examined by ultrasound, and patients with focal lesions were evaluated additionally by CT and/or MR to establish the diagnosis of HCC. We used Barcelona Clinic Liver Cancer (BCLC) staging for patients with HCC [34]. Patients included in the study had no other significant comorbidities apart from the baseline disease stated. Patients with laboratory or other data suggesting some other inflammatory disease, whether infectious or autoimmune, were not included in our cohort. Alpha-fetoprotein levels of included patients with cirrhosis, viral hepatitis, autoimmune diseases, and NAFLD were within the normal range, and there were no detected focal liver lesions by ultrasound or/ and CT scan in those patients.

We divided patients into groups according to the etiology of liver disease and decided to see viral hepatitis as one entity, as well as autoimmune liver diseases. The group of patients with cirrhosis was evaluated as one entity since further dividing into groups according to the etiology of cirrhosis resulted in a small number of patients. None of the patients in the group of liver cirrhosis had any laboratory or morphological signs of developed HCC. In the group of patients diagnosed with HCC, underlying disease also was not considered. All patients diagnosed with HCC were cirrhotic.

For the patients diagnosed with viral hepatitis, autoimmune liver disease, and NAFLD, Liver Stiffness Measurement (LSM) was performed as a surrogate of liver fibrosis stage, stages being 0-4 (F 0-4). For the purposes of our study, we have applied a "rule of five" in determining the stage of liver fibrosis [35].

All patients with HBV infection were on treatment with nucleotide/nucleoside inhibitors, while HCV patients were all PCR HCV RNA positive.

Patients were informed about the intention of conducting the study and gave written informed consent.

Interleukins measurement

Concentrations of IL-17 and IL-22 in sera were determined by Enzyme-Linked Immunosorbent Assay (ELISA) with commercially available kits for IL-17 and IL-22 (eBioscience, Affymetrix). The tests were performed according to the manufacturer's instructions. The ROC analysis determined IL-17 and IL-22 cut-off values.

Statistical analysis

Statistical analysis was performed using the MedCalc software package. Mann-Whitney U test was used to evaluate differences between samples. Spearman's rank correlation coefficient was used for correlation assessment. The Kruskal-Wallis test made comparisons of more than two independent samples. Statistical significance was set at $p<0.05$.

The Receiver Operating Curve (ROC) analysis was performed to determine the cut-off values. The quantification of IL-17 and IL-22 in the serum was achieved by constructing the standard curve.

Cox proportional hazard regression was performed in a forward fashion for univariate analysis and in a stepwise manner for multivariate analysis. Overall survival curves were calculated by the Kaplan- Meier method and compared by log-rank test.

Statistical significance was set at $p<0.05$.

Ethical statement

Written informed consent was obtained from each patient included in the study. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the Ethics Committee of our center.

Results and Discussion

Baseline patient characteristics

The study was conducted on a cohort of Croatian patients, all Caucasians.

Our study included 226 subjects, 28 healthy controls (HC), and 198 patients. The patient group consisted of 67 (34%) female and 131 (66%) male patients; the median age was 60 years (range 21-84 y) (Table 1). There were 33 patients with Hepatocellular Carcinoma (HCC), 51 with Liver Cirrhosis (LC), 43 with Viral Hepatitis (VH), 50 with Autoimmune Liver Disease (ALD), 21 with Non-Alcoholic Fatty Liver Disease (NAFLD). Underlying diseases in patients with HCC, who all had cirrhosis at the moment of HCC detection, were HBV in 9 patients, HCV in 3, alcoholic cirrhosis in 15, and there were six patients with cirrhosis of unknown origin who developed HCC. In the group of LC, there were ten patients with HCV as an underlying etiology, 7 with HBV, 32 with alcohol, and 12 with cryptogenic cirrhosis, presumably NASH. In the group ALD, there were 29 patients with Primary Biliary Cholangitis (PBC), 7 with Primary Sclerosing Cholangitis (PSC), and 10 with Autoimmune Hepatitis (AIH). All patients with HCC had LC. LSM is used as a surrogate of liver fibrosis, and mean values of liver stiffness measurements in the groups of ALD, NAFLD, and VH were 6.7, 6.4, and 8.1 kPa, respectively, representing the mean value of fibrosis of F1, F1, and F2, respectively.

All the collected clinical and laboratory data for all patients and controls are shown in Table 1.

Serum concentrations of IL-17 and IL-22

Concentrations of IL-17 and IL-22 were determined in all patients and healthy controls, and data are shown in Table 1.

The ROC curve showed the diagnostic efficiency of IL-17 and IL-22 in the differentiation between liver disease patients and the control group (p<0.0001). The ROC curve for IL-17 (Area under the ROC curve (AUC) 0,770; 95% CI 0,710 to 0,822) showed a sensitivity of 75.24 % and a specificity of 77.71 % at a cut-off value of 2.63 pg/mL. The AUC for IL-22 was 0,880 (95% CI 0,832 to 0,919) with a sensitivity of 90.12 % and specificity of 77.22% at a cut-off value of 31.6 pg/mL.

We found significantly higher concentrations of both interleukins in the patient group (IL-17 1.02-86.94 pg/mL, median 5.53: 95% CI 4.81-6.12; IL-22 14.53-786.92 pg/mL, median 55.60: 95% CI 44.38-66.31) in comparison to the healthy controls (IL-17 1.04-10.24 pg/mL, median 1.71: 95% CI 1.21-2.74; IL-22 14.73-39.41 pg/mL, median 20.25: 95% CI 18.2-27.3) (Figure 1A).

The highest concentrations of both IL-17 and IL-22 were found in patients with LC, while the lowest concentrations of IL-17 and IL-22 were found in ALD and NAFLD patients, respectively (Figures 1B and C).

No influence of age and gender on the concentration of IL-17 or IL-22 was detected across the group of patients (data not shown).

In 83% (164/198) of patients, the IL-17 value was higher than the determined cut-off value (2.63 pg/mL), and IL-22 was higher than the cut-off value (36.13 pg/mL) in 77% (152/198) of patients. Healthy controls had higher values than the cut-off values of IL- 17 and IL- 22 in 21% (6/28) and 10% (3/28), respectively.

IL-17 concentrations were higher than the cut-off value in 80% (40/50) of patients with ALD, 76% (16/21) with NAFLD, 79% (34/43) with VH, 91% (30/33) HCC and 96% (49/51) LC.

Values of IL-22 were higher than the cut-off value in 72% of patients with ALD (36/50), 76% (16/21) with NAFLD, 79% (34/43) with VH, 76% (25/33) in HCC, 88% (48/51) in LC.

When we compared the concentrations of IL-17 in patients with liver cirrhosis and other liver diseases without cirrhosis, we found statistically significantly higher levels of IL-17 in cirrhotic patients than in patients with ALD (p<0.0005), NAFLD (p<0.05), VH (p<0.005) and HCC (p<0.05) (Table 2).

Sera of the patients with HCC had statistically significantly higher concentrations of IL-17 compared to the sera of patients with ALD (p<0.0005) and lower than patients with LC (p<0.05). There was also a statistically significant difference between IL-17 concentrations in sera of patients with NAFLD and ALD (p<0.05) as well as with LC (p<0.05). Concentrations of IL-17 in sera of patients with VH were

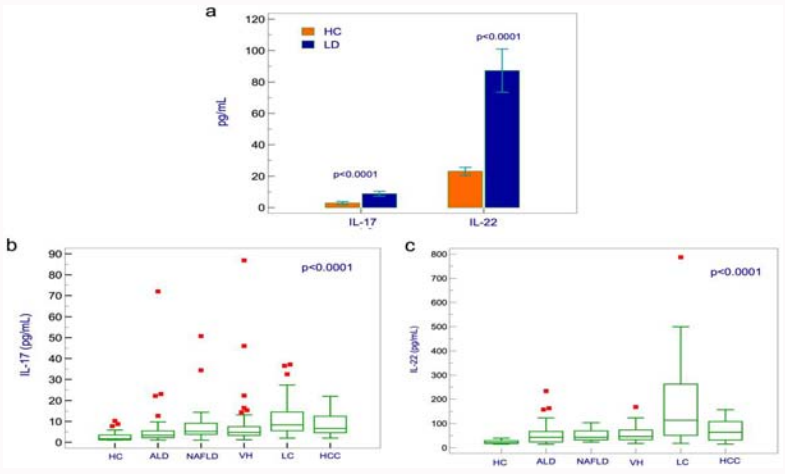

Figure 1: Concentrations of IL-17 and IL-22 proteins in sera of patients with liver diseases. (a) Summary data of serum IL-17 and IL-22 concentrations in Healthy Control (HC) and all tested Liver Diseases (LD). (b) Summary data of serum IL-17 in HC and specific liver diseases, Autoimmune Liver Disease (ALD), Non-Alcoholic Fatty Liver Disease (NAFLD), Viral Hepatitis (VH), Liver Cirrhosis (LC) and Hepatocellular Carcinoma (HCC). (c) Summary data of serum IL-17 in HC and specific liver diseases. In b and c, data are presented as 95% confidence interval (boxes), mean (line), and SEMs. Red squares indicate out-layers. Statistical significance was tested by the Kruskal-Wallis test. Post hoc analysis of statistical significance among groups in b and c is shown in Table 2.

Table 1: Demographic and clinical characteristics of patients.

				SUBJECTS GROUPS					
				HC	ALD	NAFLD	VH	HCC	LC
	N			28	50	21	43	33	51
ETIOLOGY	HCV						24	9	10
	HBV						18	3	7
	HBV+HCV						1		
	PBC				29				
	PSC				7				
	AIH				10				
	ALCOHOL							15	32
	UNKNOWN							6	2
Gender		F	N	17	24	13	16	7	7
			%	61	48	62	37	21	13
		M	N	11	26	8	27	26	44
			%	39	52	38	63	79	87
Age		Median		52	60	60	55	63	60
		Range		48-62	21-78	37-74	27-79	50-77	23-84
IL-17 (pg/mL)		Median		1.71	3.36	5.23	4.73	6.63	8.29
		Range		1.04-10.24	1.72-72.03	1.02-50.71	1.24-36.91	1.27-22.03	1.98-38.51
IL-22 (pg/mL)		Median		20.25	41.33	43.11	46.24	63.67	113.12
		Range		14.73-39.41	14.53-234.51	22.93-103.22	17.56-167.74	14.81-157.14	17.81-786.92
IL-17/IL-22		Median		0.072	0.099	0.129	0.102	0.15	0.114
		Range		0.032-0.663	0.016-0.356	0.040-0.491	0.021-1,31	0.021-0.910	0.007-0.382
AST (U/L)		Median			32	42	51	72.5	67
ref. values 11-38 U/L		Range			17-154	24-65	22-123	18-326	16-146
ALT (U/L)		Median			32	40	54	66	67
ref. values 12-48 U/L		Range			16-307	26-68	23-223	16-372	18-152
Albumin (g/L)		Median			36.3	43.5	43	33.3	28.3
ref. values 40.6-51.4 g/L		Range			23.3-41.8	38.3-48.1	37.3-48.3	21.7-43.9	21.2-48.7
AFP (µg/L)		Median						40.5	
ref. values <10 µg/L		Range						3.8-24056	
LSM (kPa)		Median			6.7	6.4	8.1		
		Range			3.8-11.5	3.3-12.5	4.1-29.2		
CAP (dB/m)		Median			280	279.5	287.5		
		Range			185-325	218-332	194-348		
CP	Number	Median						9	8
		Range						May-14	May-14
	N	A						11	18
		B						13	14
		C						9	18
BCLC	N	A						0	
		B						16	
		C						9	
		D						8	

Legend: F: Female; M: Male; N: number of subject/patients; HC: Healthy Controls; ALD: Autoimmune Liver Diseases; NAFLD: Non-Alcoholic Fatty Liver Disease, VH: Viral Hepatitis; HCC: Hepatocellular Carcinoma; LC: Liver Cirrhosis; HCV: Hepatitis C Virus Infection; HBV: Hepatitis B Virus Infection; PBC: Primary Biliary Cholangitis; PSC: Primary Sclerosing Cholangitis; AIH: Autoimmune Hepatitis; AST: Aspartate aminotransferase; ALT: Alanine Aminotransferase; AFP: Alpha-Fetoprotein, LSM: Liver Stiffness Measurement; CAP: Continuous Attenuation Parameter; CP: Child-Pugh Stage; BCLC: Barcelona Cancer Liver Clinic grade.

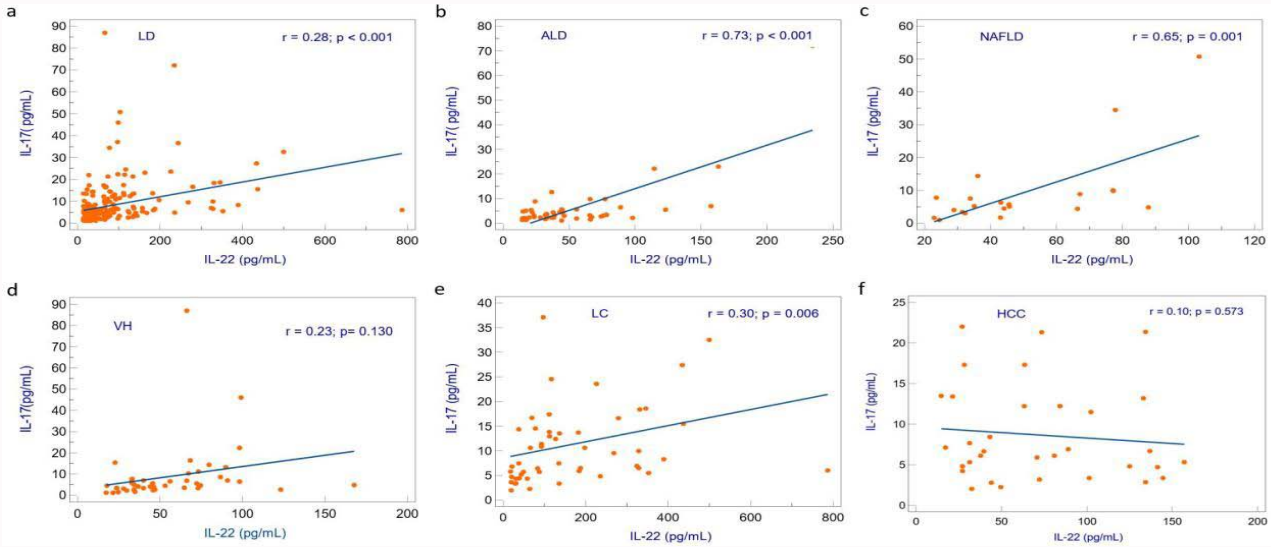

Figure 2: Correlation of serum IL-17 and IL-22 in patients with liver disease. Correlation of serum IL-17 and IL-22 in patients with liver disease of any origin (a); Autoimmune Liver Disease (ALD) (b); Non-Alcoholic Fatty Liver Disease (NAFLD) (c); Viral Hepatitis (VH) (d); Liver Cirrhosis (LC) and Hepatocellular Carcinoma (HCC) (f).

	IL-17						IL-22					
	HC	ALD	NAFLD	VH	HCC	LC	HC	ALD	NAFLD	VH	HCC	LC
HC		**	***	***	***	***		***	***	***	***	***
ALD	**		*	*	***	***	***		***	***		***
NAFLD	***	*				*	***	***				***
VH	***	*				**	***	***				***
HCC	***	***				*	***					**
LC	***	***	*	**	*		***	***	***	***	**	

*** p<0.0005 ** p<0.005 * p<0.05

Table 2: Post hoc analysis of statistical significance among specific groups of liver diseases and control.
Autoimmune liver disease (ALD), non-alcoholic fatty liver disease (NAFLD), viral hepatitis (VH), either B or C, hepatocellular carcinoma (HCC), liver cirrhosis (LC), and healthy controls (HC).

significantly different from patients with ALD and LC, p<0.05 and p<0.005, respectively (Table 2).

IL-22 was significantly higher in the sera of patients with LC compared to ALD (p<0.0005), NAFLD (p<0.0005), VH (p<0.0005), and HCC (p<0.005). There was no statistically significant difference between the concentrations of IL-22 in the sera of patients with HCC compared to ALD (p=0.0607), NAFLD (p=0.17), and VH (p<0.05). However, it was statistically significantly higher in patients with NAFLD compared to ALD (p<0.0005) (Table 2).

The correlation of IL-17 and IL-22 levels was found to be significant (p<0.001) regardless of the etiology of liver disease, meaning both interleukins were higher in liver diseases than in healthy controls (Figure 2A). The same was found when we analyzed etiologies separately for ALD (p<0.001) (Figure 2B), NAFLD (p=0.001) (Figure 2C), VH (p=0.130) (Figure 2D), LC (p=0.006) (Figure 2E). No statistically significant correlation existed between serum IL-17 and IL-22 for hepatocellular carcinoma. Still, a trend toward a negative correlation suggested a drop in IL-22 concentrations in patients with HCC (Figure 2F). The albumin and AFP concentrations, as well as the BLBC stage, were not statistically related to the concentrations of IL-17 (p=0.752; p=0.275; p=0.501) or IL-22 in HCC (p=0.248; p=0.782; p=0.368).

There was no correlation between IL-17 or IL-22 concentrations and albumin concentrations in liver cirrhosis (p=0.527; p=0.995).

According to the univariate Cox regression analyses, the following variables had a significant impact on Overall Survival (OS) in HCC patients: age (p=0.0006), ALT (p=0.0384), AST (p=0.0358) and IL-17 (p=0.0277). In multivariate analysis, only age was a statistically

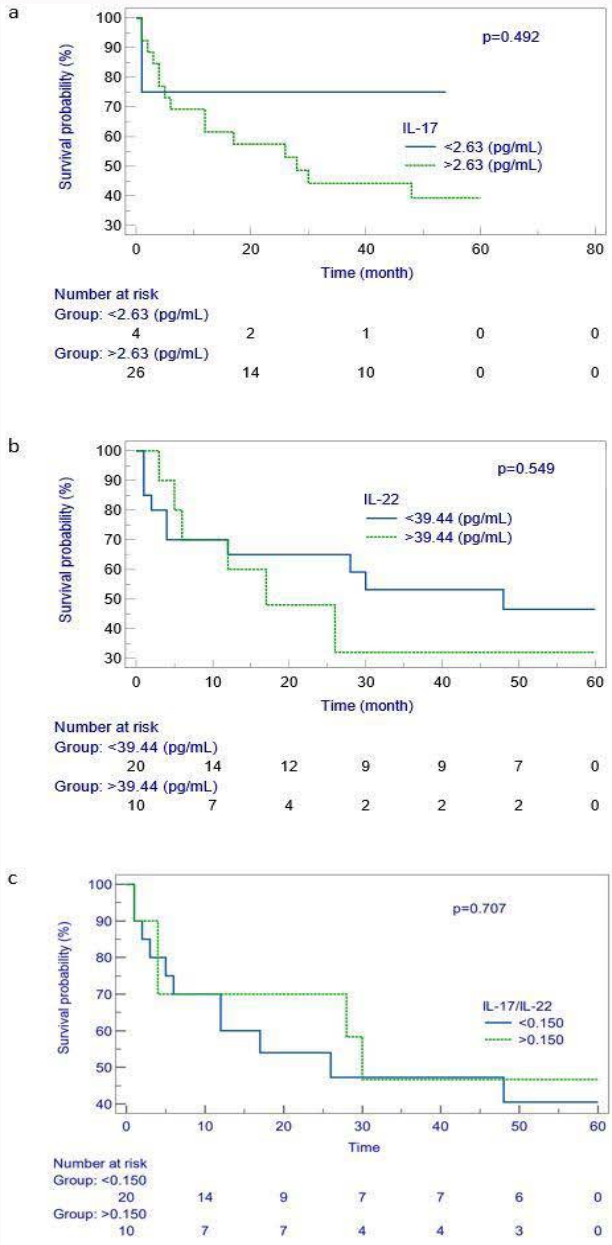

Figure 3: Stratified survival rates for HCC patients. Kaplan–Meier survival analysis of HCC patients stratified by (a) IL-17, (b) IL-22, (c) IL-17/IL-22 ratio.

significant predictor of OS (Table 3).

Kaplan-Meier analysis

Thirty patients with HCC were included in the survival analysis. The differences in the Kaplan-Meier curves for IL-17 and IL-22 were not statistically significant in patients with HCC (IL-17 p=0.492; IL-22 p=0.549). However, the curves show that patients with higher values of IL-17 and IL-22 than the cut-off values have shorter survival (Figure 3A and B).

When the analysis was done using the IL17/IL22 ratio, we also did not obtain a statistically significant difference. Still, curves do show that patients with a lower ratio have a shorter survival (Figure 3C).

Discussion

Our results show that both interleukins are significantly higher in patients with liver disease than in healthy controls. Those findings are in accordance with so far published data and are expected, given the previously discussed role of IL-17 and IL-22 in maintaining tissue homeostasis [11,13,36,37]. The highest concentrations of IL-17 and IL-22 were found in patients with liver cirrhosis.

Similar findings for concentrations of IL-17 were published by Liang et al. in 2021 [2], where they found IL-17 to indicate imminent HCC in liver cirrhotic patients. Given the dual role of IL-17, high concentrations of IL-17 in the cirrhotic liver may result in a strong inflammatory but still protective response in the liver tissue. High concentrations of IL-22 could be due to the same reason, supported by the Kronenberger findings published in 2012 [38], stating the possibility of predicting the severity of cirrhosis by the concentrations of IL-22 in serum. In our cohort of LC patients, there was a statistically significant correlation between CP status and IL-22 but not IL-17. Those findings are in accordance with Kronenberger's and some other authors' findings [39]. Our results showed a strong correlation between IL-17 and IL-22 in all investigated entities except hepatocellular carcinoma.

While there was still a significantly higher concentration of IL-17 and IL-22 in hepatocellular carcinoma than in healthy controls, those concentrations were significantly lower than in cirrhotic patients. Thus, we hypothesize that changes in the concentration of these interleukins might be important to identify patients who developed HCC. Furthermore, in the patients with developed HCC in cirrhotic liver, and our HCC group consisted of patients with cirrhosis, there was observed a trend toward a negative correlation of CP stage and IL-22 concentration. This could imply that cirrhosis itself is not the reason for the drop in IL-22 concentration but the development of HCC. Inflammation has been defined as enabling characteristic of cancer [40]. Complex interaction among components of inflammation including secretion of cytokines may favour tumor development. However, changes in transcription regulation may develop along with tumorigenesis.

Future studies are necessary to elucidate the role of IL-17 and IL-22 in developing HCC in patients with LC. Although statistically non-significant, the separation of overall survival curves between low and high IL-17 and IL-22 suggests that higher concentrations of these cytokines in HCC reflect the more aggressive nature of the tumor.

We also observed a possible trend toward a negative correlation between IL-17 and IL-22 in hepatocellular carcinoma. This result warrants further investigation on a larger patient cohort.

In our cohort of patients, we did not find any correlation between investigated interleukins concentrations with fibrosis stage, represented by liver stiffness measurements, nor did we find a correlation with albumin concentration. Those findings are probably influenced by the size of the patient's groups since it could be expected to find a rise in interleukins concentrations with the progression of fibrosis toward cirrhosis [41,42].

Regarding survival, the follow-up period in our study was 30 months (range 1-60). We found no statistically relevant correlation between IL-17 and IL-22 concentrations and survival. However, we observed that a proportion of patients with HCC with lower concentrations of IL-17 or IL-22 showed a trend for more prolonged survival than patients with higher concentrations of these cytokines. Results of studies that correlated survival and concentration of these cytokines vary. Lee et al. conducted a study on 114 patients with HCC

Table 3: Univariate and multivariate Cox regression analysis of prognostic factors to predict overall survival in HCC patients.

Univariate						
Variable	Coefficient	Standard Error	Wald Chi-Square	p	Risk Ratio	95% Confidence Interval
Gender	0.814	0.939	0.753	0.385	2.258	0.359-1.215
Age	0.052	0.077	0.454	**6E-04**	0.906	0.906-1.1224
ALT	-0.056	0.027	4.2879	**0.038**	0.945	0.895-0.997
AST	0.066	0.0354	3.457	**0.036**	1.068	0.996-1.145
IL-17	-0.176	0.121	2.118	**0.028**	0.838	0.661-1.0631
IL-22	-0.008	0.008	1.057	0.304	0.991	0.975-1.008
Multivariate						
Variable	Coefficient	Standard Error	Wald Chi-Square	p	Risk Ratio	95% Confidence Interval
Age	0.116	0.04	8.451	**0.036**	1.123	1.029-1.215

with a follow-up of 60 months and found that patients with detectable IL-22 exhibited better overall survival [39]. A study by Liang et al. included 404 patients with HCC and found that patients with higher IL-17 concentrations had shorter survival [2]. Since our cohort was smaller, there is a need for further investigation of a larger cohort of patients.

This study was performed on a Caucasian, European, Croatian population, which is one of its values since most studies regarding HCC are done on Asian populations.

One of the most significant limitations of our study was the number of patients when we divided the groups according to the underlying etiology of liver disease.

Patients with viral hepatitis made one etiology group. Still, since there were patients with HVB on treatment and HCV patients were without treatment at the time of sampling blood, this could also influence the results.

Finally, our group of patients with HCC consisted of patients in BCLC stage B-D. No patients were in stage 0 or A, which could have influenced our findings.

Conclusion

We found significant differences in serum IL-17 and IL-22 concentrations between patients with liver diseases and healthy controls. Even more interesting finding was the significant difference in serum IL-17 and IL-22 concentrations between patients with liver cirrhosis and HCC. These two interleukins may represent potential biomarkers that could be used to identify patients with LC who have developed HCC. More extensive studies are required to validate IL-17 and IL-22 serum concentrations as novel markers for early HCC detection.

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